30. (New) A method according to claim 14, wherein said denaturing agent comprises formamide.

REMARKS

Reconsideration of this application is requested. The claims presented for reconsideration are claims 1-29. New claims 24-30 correspond to the subject matter removed from some of the amended dependent claims.

Claims 1-23 were rejected under 35 USC 112, second paragraph. Claims 1, 6-7, 10-14 and 16-18 were amended. In light of these claim amendments, Applicant respectively requests withdrawal of this rejection. Informalities to the remaining claims previously presented have also been corrected.

With regard to some of the 112, second paragraph rejections, Applicant's note that it is proper in a dependent claim to recite an element in an independent claim without repeating all of the descriptive language associated with that element. For example, if the independent claim recites to "at least one specific probe" or to "whole cells", it is not necessarily indefinite to use the term "the probe" or "cells", respectively, in a dependent claim. However, if there were two different types of probes recited in the claim, e.g., a specific probe and a universal probe, use of the term "the probe" in a dependent claim would be indefinite under 112, second paragraph because it would not be certain as to which probe was being referred to.

Claims 1-23 were rejected under 35 USC 103(a) as being unpatentable over the combination of Manz et al., Wagner et al., De Los Reyes et al. in view of Marbarry. Claim 1 was amended in accordance with Examiner's suggestion beginning near the middle of page 7 of the Office Action. Claim 1 was amended to better define the extraction and separation of the hybridized probes from the cells for quantitative detection.

In view of the above, consideration and allowance are respectfully solicited. Attached hereto is a marked-up version of the changes made to the specification and

claims by this amendment. The attached page is captioned "**Version with Markings to show changes made**

In the event the Examiner believes an interview might serve to advance the prosecution of this application in any way, the undersigned attorney is available at the telephone number noted below.

The Director is hereby authorized to charge any fees, or credit any overpayment, associated with this communication, including any extension fees, to Deposit Account No. 22-0185.

Respectfully submitted,

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Date: 12/06/01

Version with markings to show changes made

IN THE CLAIMS

Claims 1-23 have been amended as follows:

1. (Amended) A method of qualitative and quantitative analysis of [the] microbial population(s) [potentially present in a sample, characterized in that it comprises] comprising:

[-]contacting [the] microorganisms [potentially] present in [said] a sample with at least one [RNA-targeted oligonucleotide probe, hereafter called] specific probe to form a probe-target complex, [able to target a desired microbiological population,] wherein the specific probe recognizes a RNA target sequence under conditions [favourable] favorable to *in situ* hybridization in whole cells,

[-]extracting the specific probes that are hybridized by separation from their target [and elution outside said cells those probes which have become hybridized], and

[-]detecting the extracted probes and measuring the amount thereof or their respective amounts.

2. (Amended) A method according to Claim 1, wherein [further characterized in that] said at least one specific probe is chosen among the group consisting of Nb 1000 (SEQ ID N°1) and Nso 1225 (SEQ ID N°2).

3. (Twice-Amended) A method according to Claim 1, further [characterized in that] comprising contacting said microorganisms [potentially] present in said sample [are contacted with another probe, hereafter called] with an universal probe[, serving] to normalize results [obtained with probes targeting specific phylogenetic groups of microorganisms].

4. (Amended) A method according to Claim 3, [further characterized in that] wherein said universal probe is chosen among the group consisting of S Univ-1390 SEQ ID N°3) and S Bac 338 (SEQ ID N°4).

5. (Twice-amended) Method according to Claim [1] 3 [further characterized in that] wherein said specific [and/or] or said universal [probe(s) is a (are)] probe is a *r*RNA-targeted [probe(s)] probe.

6. (Twice-amended) A method according to Claim 1, [further characterized in that] wherein said microorganisms [potentially present] in said sample are extracted from said sample[, notably] by centrifugation.

7. (Twice-amended) A method according to Claim 1, [further characterized in that] wherein said contacting is performed followed by fixation of said whole cells.

8. (Amended) A method according to Claim 7, [further characterized in that said] wherein fixation of the cells is achieved by incubation of [said] the cells in a solution of less than 10% paraformaldehyde[, preferably about 4%,] for 3 to 12 hours at 4 °C.

9. (Twice-amended) A method according to Claim 7, [further characterized in that] wherein said fixation is followed by a dehydration step, prior to said contacting step.

10. (Amended) A method according to Claim 9, [further characterized in that said] wherein the dehydration [step] is performed by placing said sample in contact with at least one ethanol solution[, and preferably with a series of ethanol solutions of increasing concentrations].

11. (Twice-amended) A method according to Claim 1, [further characterized in that said] wherein said contacting is performed by placing said sample in contact with

said [at least one] specific probe in the presence of a hybridization solution[, hereafter called hybridization solution, which notably comprises] comprising a denaturing agent [such as sodium dodecyl sulfate (SDS)] at a concentration [in a 0,000-0,1% range, preferably on the order of 0.01%] of from 0.001% to 0.1%, Tris-HCl[,] with a pH of about 8 at a concentration [in a 0,001-0,1 M range, preferably on the order of 0.02M] of from 0.001 M to 0.1 M; and a salt [such as sodium chloride] at a concentration [in a 0,1-1,5 M range, preferably on the order of 0.9 M] of from 0.1 M to 1.5 M.

12. (Twice-amended) A method according to Claim 1, [further characterized in that said] wherein contacting [phase] is performed for an incubation time of about 10 minutes to about 2 hours, and at [the] an optimal hybridization temperature.

13. (Twice-amended) A method according to claim 1, [further characterized in that said] wherein [extraction] extracting of said [at least one] specific probe is performed following removal of [the] excess and unbound specific probe or of non-specifically associated probe material [placed in contact, notably by washing with a solution, hereafter called wash solution, which notably comprises] by contacting with a wash solution comprising a denaturing agent [such as sodium dodecyl sulfate (SDS)] and a salt [such as sodium chloride] at concentrations appropriate for achieving the stringency necessary [to] for the removal of non-specifically associated probe.

14. (Twice-amended) A method according to Claim 1, [further characterized in that said] wherein [extraction is performed by placing said microorganisms potentially present under conditions enabling the denaturation of every all probe specifically associated with its target sequence, notably in the presence of an agent able] extracting of the hybridized probes includes adding a denaturing agent to denature the probe-target [duplex] complex, and at a temperature higher than the melting temperature of the specific probe under consideration[, notably at a temperature of approximately 100°C].

15. (Amended) A method according to Claim 14, [further characterized in that] wherein the denaturing agent is formamide.

16. (Twice-amended) A method according to Claim 1, [further characterized in that] wherein said extracted probes are concentrated[, notably using the Speed-Vac®,] prior to the measurement of the amount thereof or of their respective amounts.

17. (Twice-amended) A method according to Claim 1, [further characterized in that] wherein said [detection] detecting and amount measurement of the extracted probes [is performed by] includes detection and amount measurement of a label associated or incorporated into [each of the contacted] the extracted probes, [such as a] wherein the label is a radioactive, chemiluminescent or fluorescent label[, notably such as fluorescent].

18. (Twice-amended) A method according to claim 1, [further characterized in that] wherein said sample is taken from fluids [such as] selected from natural water, industrial water, industrial effluent, municipal wastewater, industrial sludge, thermal mud, food liquid or gel, fermentation media, air, gas, aerosol, [or is] a sample taken from a building ventilation duct[,] or air conditioning duct, a sample of food solid, a sample of soil, a sample from medical apparatus, or is a human or animal sample[, such as] selected from blood, urine, vaginal or intestinal flora.

19. (Twice-amended) A method according to Claim 1, [further characterized in that] wherein it is used in combination with a process for triggering an alarm in connection with quality, safety and/or sanitary monitoring of the product from which said sample has been obtained.

20. (Twice-amended) A method according to Claim 1, [further characterized in that] wherein it is used in *in vitro* diagnosis of an infectious disease.

21. (Twice-amended) A method according to Claim 1, [further characterized in that] wherein it is used in the automatic or feedback control of a microbiological process such as methane fermentation of liquid manure, treatment of organic effluents, sewage treatment process such as treatment by activated sludge.

22. (Twice-amended) A method according to Claim 1, [further characterized in that] wherein it is used in the automatic or feedback control of a process relating to the removal or prevention of the development of microorganisms.

23. (Twice-amended) A method according to Claim 1 [further characterized in that] wherein it is applied in the detection of foam formation during the implementation of activated sludge processes and/or the feedback control of a method relating to the removal or prevention of the said foams.